GRASPING THE STRUCTURE OF INSECT MUSCLE POISED TO CONTRACT

Researchers have achieved the first detailed view of resting muscle filaments poised to contract, a long sought window into the biochemical cycle that causes muscle contraction. The group determined the overall structure of insect muscle fibers from x-ray diffraction patterns and performed computer modelling to analyze the data. The resulting structure, reported in August 2003, suggests a specific mechanism for insect muscle activation.

Muscle cells (fibers) contain two parallel, overlapping sets of protein filaments, made of myosin and actin, respectively, that align with the direction of contraction. The myosin and actin filaments have fixed length individually, but they slide past each other like telescope segments to stretch or shorten the overall muscle. The shortening motion is driven by comma-shaped myosin "heads," which radiate out from the myosin filaments and cyclically bind to, bend against, and then release adjacent actin filaments. The bind-and-bend phase of each cycle is called a powerstroke. Comparing bound and resting head structures and positions can help researchers deduce the powerstroke mechanism. Researchers had previously viewed the end state of the powerstroke, in which myosin heads have bent and are strongly bound to actin (Fig. 1). No one had reported the orientation of the unbound heads in myosin's relaxed initial state until August 2003, however.

Researchers from Imperial College London, Duke University, and the Illinois Institute of Technology decided that improved synchrotron technologies and data analysis tools offered the prospect of modelling the relaxed structure. They recorded so-called low-angle x-ray diffraction patterns from flight muscle fibers of giant waterbugs on the Bio-CAT beamline 18-ID at the APS, which gave them new and sharper details of the large-scale myosin filament structure. A single recording cannot produce both large- and fine-scale data because large structural elements are encoded in low-angle features of the diffraction pattern and vice versa; cameras can resolve one or the other, but not both. So the group computed thousands of different ways of fitting existing high-resolution atomic models of head structure into the overall model, on the basis of the assumption that each head could pivot at two points, and identified the best fit to the observed low-angle x-ray pattern. The model confirmed that resting myosin filaments have stacked rings or "crowns" of eight heads each — two heads per myosin molecule. One head of each myosin projects about 90° from the filament axis; the other tucks inward against the filament's circumference.

Comparing the results of modeling against actual post-powerstroke myosin head conformations in waterbug and many other species suggests the changes that take place during a powerstroke cycle (Fig. 1). Each of the four projecting heads rotates slightly about the "neck," which connects to a pivot on the myosin filament. The rotation positions the head to bind tightly to the actin filament and tilt forcefully by 45° relative to the

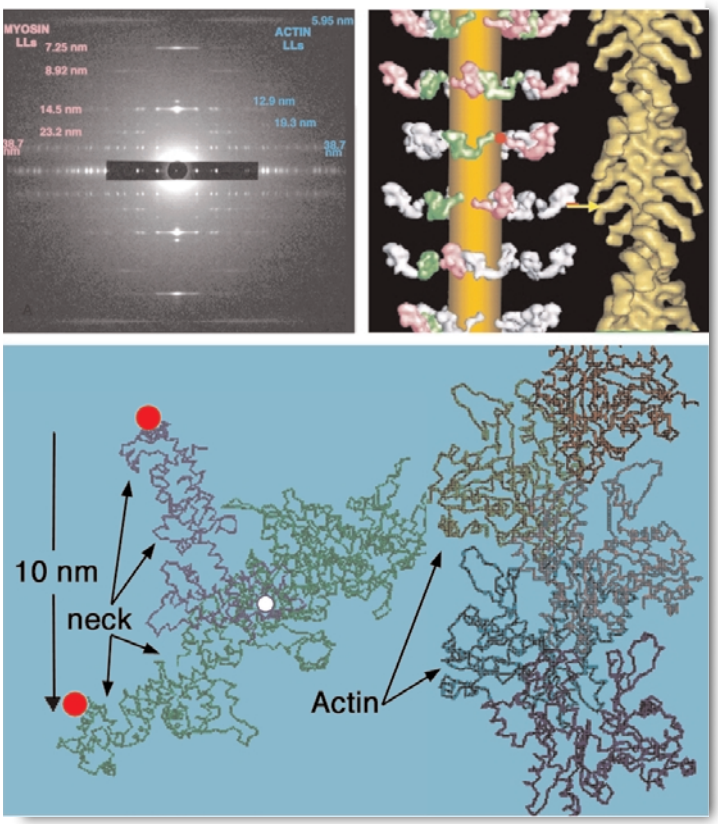

Fig. 1. Top left: Low-angle x-ray diffraction pattern from relaxed insect flight muscle. (Pink and blue numbers represent spacings between repeating structural elements as calculated from the pattern.) Top right: Structure (left) of the modelled myosin heads (pink/white/green) in their relaxed, pre-powerstroke state and (right) in their "rigor" or post-powerstroke state. Bottom: Transition from the head shape in relaxed insect muscle (blue) to rigor-like final shape (green), showing the pivots needed (red on thick filament, white within head) to let the neck swing from one form to the other when bound to actin.

axis of the filament. Such a powerstroke would move the head and bound actin filament 10 nanometers down the axis. The four inward-pointing heads each touch an adjacent projecting head. These contacts may restrain both myosin heads from cleaving high-energy ATP molecules—the powerstroke fuel—until the right moment.

Insect flight muscles must contract rapidly and precisely to keep the insect aloft. As if optimized to do so, the relaxed outward myosin heads seem poised to bind actin and begin powerstroking. The researchers speculate that the increased calcium in stimulated muscle cells may loosen the heads and modify actin filaments to facilitate binding. Stretching flight muscle triggers concerted powerstrokes, perhaps by perturbing the ring of contacts between inward and outward heads to fully enable ATP splitting and actin binding. ❍

See: H.A. Al-Khayat[1], L. Hudson[1], M.K. Reedy[2], T.C. Irving[3], and J.M. Squire[1], "Myosin Head Configuration in Relaxed Insect Flight

Muscle: X-ray Modeled Resting Cross-Bridges in a Pre-power-stroke State Are Poised for Actin Binding," Biophys. J. **85**, 1063–1079 (August 2003).

Author affiliations: [1]Imperial College London, [2]Duke University, [3]Bio-CAT/Illinois Institute of Technology

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METAL SENSOR KEEPS COPPER IN CHECK

How's this for a hair trigger? A metal-sensitive molecular switch found in *E. coli* bacteria trips at a zeptomolar concentration of free copper—equivalent to one free copper ion in every 10^{11} cells. Researchers determined the three-dimensional structure of the protein switch, which helps maintain copper levels in the cell, and found that an unusual binding pocket allows it to distinguish copper from other metal ions with such spectacular sensitivity.

Metal ions are crucial to many enzymes, but too much metal floating around in a cell may weaken or kill it. A family of proteins sensitive to zinc, mercury, and other metal ions therefore monitors the cell interior and turns on ion pumps, detoxifying enzymes and other cellular machinery if metal concentrations get too high. If more than a few thousand copper ions accumulate in a cell, for example, the CueR protein senses the change and takes action. To test the sensitivity of CueR, researchers from Northwestern University added decreasing amounts of copper to a mixture of this sensor protein and one of the genes it activates, called copA. Active, copper-bound CueR binds to the copA DNA and recruits enzymes to copy the gene into molecules of RNA. The mixture began producing RNA at a tiny concentration of copper, corresponding to one free ion in a volume the size of 10^{11} *E. coli* cells.

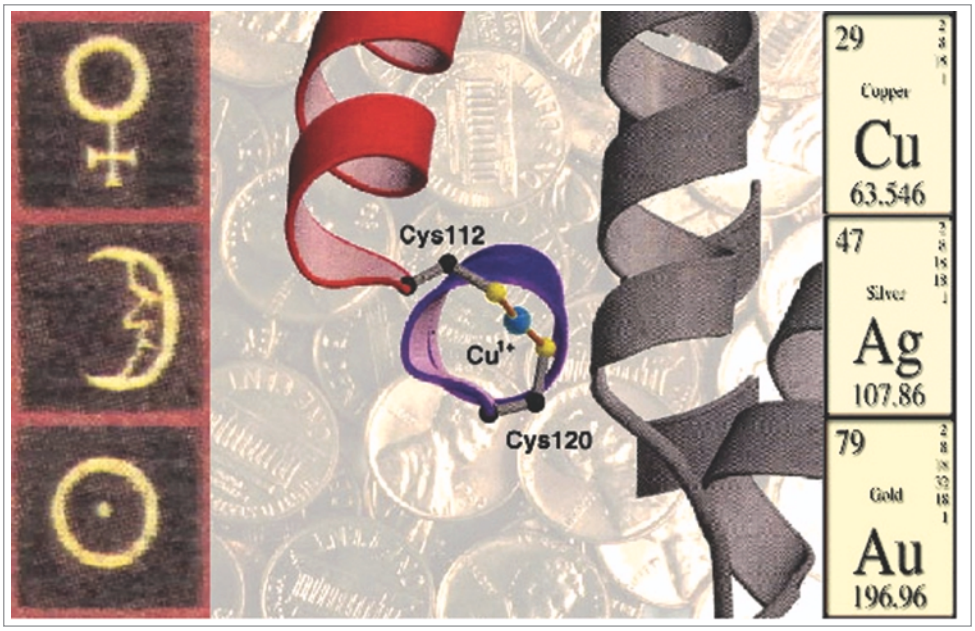

Fig. 1. The CueR protein is spectacularly sensitive and specific to metal ions with one unit of positive charge—including copper, silver and gold—despite a relatively simple linear binding geometry. Here two sulfur atoms (yellow) hold a copper ion (blue) in place.

CueR achieves its sensitivity and specificity by burying a copper ion away from solution and spearing it between two sulfur atoms (Fig. 1). The group compared the structure of CueR, which recognizes metal ions that have only a single unit of positive charge, to that of the zinc sensor ZntR, which recognizes only ions that have two units of charge, such as zinc . The researchers crystallized both metal sensors and solved their structures from x-ray diffraction patterns, obtained at the DND-CAT 5-ID beamline at the APS, and the Stanford Synchrotron Radiation Laboratory in Stanford, California. To confirm that the copper ion and sulfur atoms are arranged in a line, the Northwestern researchers and a group from the University of Michigan in Ann Arbor scattered x-rays of increasing energy off the protein. The energy of the resulting photons emitted from the copper-sulfur complex were consistent with a linear geometry.

The structure reveals several reasons for the specificity of CueR. Ions with less charge prefer to form fewer bonds with adjacent atoms. The cramped metal binding pocket of CueR is structured to offer an ion only the two sulfur atoms for bonding, whereas ZntR holds two zinc atoms in a web of four bonds each. CueR also stabilizes the copper-sulfur complex by neutralizing its net negative charge. Each sulfur atom is negatively charged. The copper ion neutralizes one negative charge, and an adjacent protein dipole offsets the other. A greater local charge would upset that balance in the greasy interior of the protein.

The surprisingly simple linear arrangement suggests that the sensitivity of CueR rises from the charge neutralization and additional weak bonding between copper and the surrounding pocket of amino acids, the researchers say. The linear copper-sulfur geometry has been reported in only a few copper-transporting proteins related to copA. The high sensitivity of CueR

also implies that the internal space (cytosol) of *E. coli* is typically starved for copper, which raises the question of how the cell supplies other molecules with the metal. One possible explanation is that copper ions are not tolerated in the cytosol, but when they overflow into it, they are transported out to the cell envelope, where all of *E. coli*'s known copper-dependent molecules are located. ❍

See: A. Changela, K. Chen, Y. Xue, J. Holschen, C.E. Outten, T.V. O'Halloran, and A. Mondragón, "Molecular Basis of Metal-Ion Selectivity and Zeptomolar Sensitivity by CueR," Science **301**, 1383-1387 (5 September 2003).
Author affiliation: Northwestern University

See also: K. Chen et al., "An Atypical Linear Cu(I)-S_2 Center Constitutes the High-Affinity Metal-Sensing Site in the CueR Metalloregulatory Protein," J. Am. Chem. Soc. **125**, 12088-12089 (2003).

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SAXS Studies Reveal Metals in the Jaws of Marine Worms

What makes our hard tissues—especially our teeth and jaws—so hard? In higher-order organisms, particularly in vertebrates (like us), it's calcium, but in some invertebrates, such as insects and worms, the fangs and mandibles contain high levels of the transition metals zinc, copper, iron, and manganese. Using the x-ray absorption spectroscopy(XAS) station on the PNC/XOR beamline 20-ID at the APS, collaborators from the University of California, Santa Barbara; Vienna University of Technology; the Austrian Academy of Sciences; Helsinki University of Technology; and Argonne National Laboratory studied the detailed composition of worm jaws, delineating the location of metal and elucidating the fundamental underpinnings of jaw strength.

In particular, two invertebrates were studied in detail: clam worms (*Nereis limbata*) and bloodworms *(Glycera dibranchiata*), both the size of a typical earthworm (about 20 cm in length). Jaws dissected from the worms were about 5 mm long or less and mainly proteinaceous, containing significant amounts of the amino acids glycine and histidine with a few weight percent metal concentrated at the tip of the jaw. The type of transition metal depends on the species: the jaws of the raptorial bloodworm contain predominately copper as the major inorganic constituent, whereas its scavenger cousin the clam worm apparently prefers zinc.

Intact worm jaws were subjected to synchrotron microbeam analysis—x-ray diffraction and x-ray absorption

Fig.1. The jaw of a *Nereis limbata worm*. The jaw is about 5 mm long, hollow at the base and solid at the tip. Higher x-ray absorption is found at the tip, indicating that that area has higher local electron density. All of the zinc is concentrated in the tip region of the jaw, with no or very low levels of zinc at the base.

experiments, done at the APS, as well as small-angle x-ray scattering.

The zinc in the *Nereis* jaw was found to be in nonmineralized form: noncrystalline, distributed in the proteineous jaw matrix, yet still hardening the structure. Nanoindentation was carried out to examine the local mechanical properties of the jaw materials and to relate the hardness and stiffness to the local metal content. A small diamond tip in the shape of an inverted pyramid was used to press on the surface of the (typically dried) jaw tissue, rendering a displacement point typically one micrometer in size. Recording of a load-displacement curve provided for the calculation of local jaw stiffness.

The presence of zinc and chlorine in *Nereis* jaws was found to correlate strongly with the local hardness and stiffness of the material. Despite the co-localization of the two elements, no simple zinc-chlorine compound was found. Instead, it is proposed that both zinc and chlorine bind directly to the protein, thus cross-linking the protein molecules and adding strength to the jaw. This idea gets extra support from the fact that the jaw protein contains a significant amount of the amino acid histidine, which prefers to coordinate with metals.

In general, jaws of *Nereis* worms were considerably softer than those of their relatives *Glycera*. The difference appears to be due to a difference of functionality: *Glycera* is a venomous species that must grasp tightly in order to inject venom, while *Nereis* jaws serve mainly to grasp small pieces of food. Form, once again, follows function. ❍

See: H.C. Lichtenegger[1,2], T. Schoberl[3], J.T. Ruokolainen[4], J.O. Cross[5], S.M. Heald[5], H. Birkedal[1], J.H. Waite[1], G.D. Stucky[1], "Zinc and mechanical prowess in the jaws of *Nereis*, a marine worm," Proc. Natl. Acad. Sci. U.S.A. **100** (16), 9144-9149 (5 August 2003).

Author affiliations: [1]University of California, Santa Barbara, [2]Vienna University of Technology, [3]Austrian Academy of Sciences, [4]Helsinki University of Technology, [5]PNC/XOR

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MAPPING THE UPTAKE OF PLATINUM ANTICANCER AGENTS IN INDIVIDUAL HUMAN OVARIAN ADENOCARCINOMA CELLS

Cisplatin, a platinum-based chemotherapeutic drug, has been a boon to physicians in treating testicular and ovarian cancer. The cisplatin analogue Pt103 is even better, because it remains active long after such tumors develop a resistance to cisplatin. This advantage has prompted research into finding the reasons why. The most critical aspect of this work involves establishing whether Pt103 differs significantly from its parent drug in cellular uptake and intracellular distribution.

Researchers from five Australian research centers—La Trobe University, the Peter MacCallum Cancer Institute, the University of Melbourne, Monash University, and the Australian Nuclear Science and Technology Organisation—explored this issue by using the state-of-the-art hard x-ray microprobe (XMP) at the XOR 2-ID-D beamline of the APS. The study aimed to determine whether the XMP is capable of quantifying the uptake and intracellular distribution of potential anticancer agents within individual adenocarcinoma cells. This capability would be a great improvement over current techniques, which obtain such information only as averages over millions of cells, because of the minute amounts of optical fluorescence marker material that become distributed within each cell.

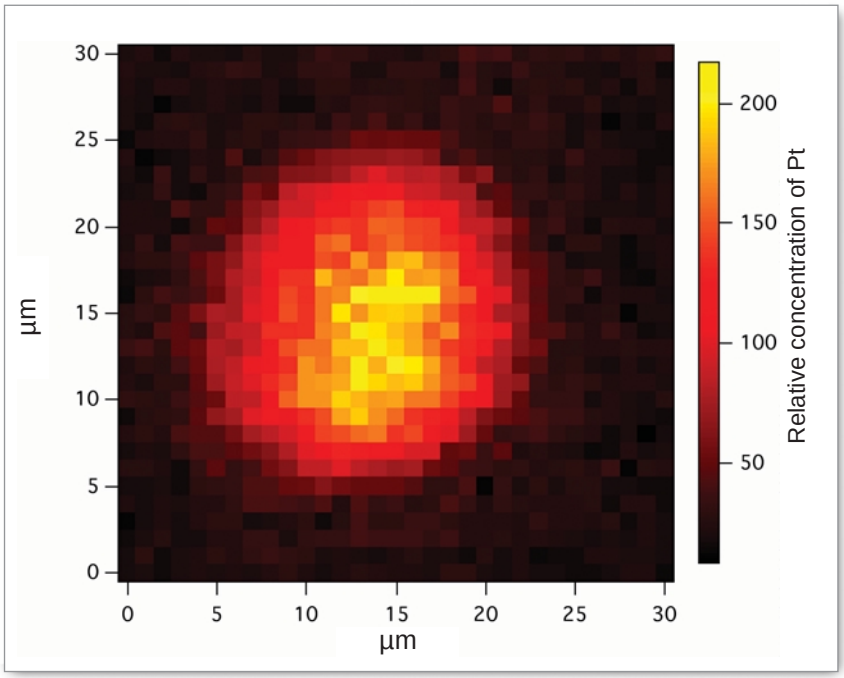

Fig. 1. The elemental distribution of platinum in a human ovarian 2008 cell that was dosed with 10 µm Pt103 over an 8-h period.

Human ovarian 2008 cells and cisplatin-resistant 2008/CDDP cells were treated with cisplatin or Pt103, snap frozen in isopentane, and then freeze-dried before being mounted on an X-Y translation stage at 2-ID-D. The samples were scanned across a microfocused x-ray spot, yielding spatially resolved fluorescence maps of many different elements simultaneously. This work mainly concentrated on the presence of platinum (Pt). An incident x-ray energy of 11.7 keV was used to excite the Pt L fluorescence lines. At this energy, x-rays penetrate biological cells without significant absorption; thus, no specimen thinning is required, and the fluorescence map of an element represents a two-dimensional projection of its volumetric distribution within the specimen.

Uptake of cisplatin in cancer cells that are resistant to the drug was found to be reduced by ~50%, compared with uptake in normal cancer cells over a 24-h period. In contrast, resistant cells showed an increased uptake of the Pt103 derivative by up to an order of magnitude. The researchers speculate that the enhanced uptake of Pt103 may allow the drug to overcome the resistance mechanism induced by long-term exposure to cisplatin. Interestingly, Pt103 uptake was found to be largest in resistant cells (over a 24-h period), which was contrary to findings from previous bulk measurements. This result highlights the difference between measurements performed on millions of cells as opposed to those performed on individual cells, as it appears from this work that measurable variation in uptake can occur within cell batches.

This experiment successfully demonstrates the feasibility of using the XMP for single-cell analysis, even for clinical doses of anticancer agents, which has not been possible previously with other analytical techniques. The XMP technique was shown to provide a minimum detectable limit of 20 attograms (2×10^{-17} grams or 6×10^{4} atoms) for platinum.

In this study, a step size of 1 μm was chosen to map out the Pt distribution in the whole cell (see Fig. 1). The XMP, however, is capable of achieving a spatial resolution of ~100 nm and may therefore be used for subcellular imaging in future studies when cell sections are used. This high spatial resolution combined with an ultimate elemental sensitivity at the parts-per-billion level pave the way for quantitative submicron three-dimensional mapping of elemental distributions within individual cells. The spatial and elemental resolution would allow for direct quantification of drug uptake in the nuclear and mitochondrial components. Such subcellular information may one day allow the detailed mechanisms of drug uptake to be studied directly, which could lead to more effective drug designs. ❍

See: P. Ilinski[1], B. Lai[1], Z. Cai[1], W. Yun[1], D. Legnini[1], T. Talarico[2,3], M. Cholewa[4], L.K. Webster[3], G.B. Deacon[5], S. Rainone[3], D.R. Phillips[2], and A.P.J. Stampfl[1,6], "The Direct Mapping of the Uptake of Platinum Anticancer Agents in Individual Human Ovarian Adenocarcinoma Cells Using a Hard X-ray Microprobe," Can. Res. **63**(8), 1776-1779, (15 April 2003).

Author affiliations: [1]Argonne National Laboratory, [2]La Trobe University, [3]Peter MacCallum Cancer Institute, [4]University of Melbourne, [5]Monash University, [6]Australian Nuclear Science and Technology Organisation

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